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(71) Applicants:
- **SHANGHAI INSTITUTE OF MATERIA MEDICA, CHINESE ACADEMY OF SCIENCES**
  **Shanghai 200031 (CN)**
- **The Garvan Institute Of Medical Research, Australia**
  **Sydney, New South Wales 2010 (AU)**

(72) Inventors:
- **YE, Yang**
  **Shanghai 200031 (CN)**
- **JAMES, David, E**
  **Sydney, New South Wales 2010 (AU)**
- **TAN, Minjia**
  **Shanghai 200031 (CN)**
- **COONEY, Gregory, J**
  **Sydney, New South Wales 2010 (AU)**
- **KE, Changqiang**
  **Shanghai 200031 (CN)**
- **KRAEGEN, Edward, W**
  **Sydney, New South Wales 2010 (AU)**
- **CHEN, Tong**
  **Shanghai 200031 (CN)**
- **YE, Jiming**
  **Sydney, New South Wales 2010 (AU)**
- **LI, Xiqiang**
  **Shanghai 200031 (CN)**

(74) Representative: **Kador & Partner**
**Corneliusstrasse 15**
**80469 München (DE)**

# (54) USE OF COMPOUNDS EXTRACTED FROM MOMORDICA CHARANTIA L. IN THE MANUFACTURE OF MEDICAMENTS FOR PREVENTION AND TREATMENT OF DIABETES AND OBESITY

(57) The present invention disclosed a medical use of cucurbitane triterpenoids represented by the following formula and isolated from *Momordica charantia L.* of Cucurbitaceae family in the preparation of medications for prevention and treatment of diabetes and obesity. The above cucurbitane triterpenoids may be acted as a glucose uptake stimulator, an agonist for the translocation of glucose transporter 4(GLUT4) to the cell membrane, and an activator of adenosine monophosphate-activated protein kinase (AMPK). They may have potential for the prevention and treatment of diabetes and obesity.

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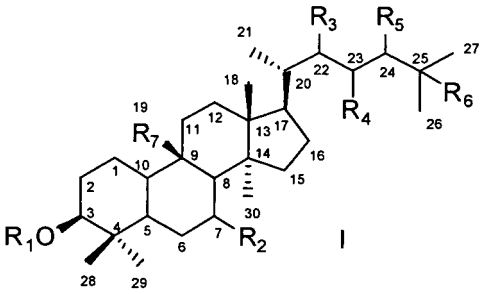
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## Description

### Technical Fields

**[0001]** The present invention belongs to pharmaceutical chemistry field, and more particularly, relates to the use of cucurbitane triterpenoids extracted from *Momordica charantia L.* and pharmaceutical compositions thereof for prevention and treatment of diabetes and obesity.

### Background of the Art

**[0002]** At present, there are more than 150 millions people suffering from diabetes worldwide and it is expected that this figure will be over 300 millions by 2025. Among them, predominant are the patients suffering from type 2 diabetes (T2D). Since insulin resistance is the main metabolic abnormality of T2D, there is of considerable interest in the development of insulin-sensitizing agents, which treat diabetes by improving insulin resistance. Two major pathways have been targeted by clinical medications to ameliorate insulin resistance: peroxidsome-proliferator-activating receptors (PPARs) and AMP-activating receptors (AMPK). Thiazolidinediones (TZDs) and biguanides are the two most generally used agents for treating diabetes at present. TZDs are widely used but can result in many adverse effects, such as weight gain, fluid retention and heart failure. The dimethyl biguanide does not result in weight gain, but mainly acts in liver rather than muscles, and thus is not a satisfied therapy for treating diabetes. Therefore, there is a worldwide search for a better insulin-sensitizing agent for the treatment of diabetes.

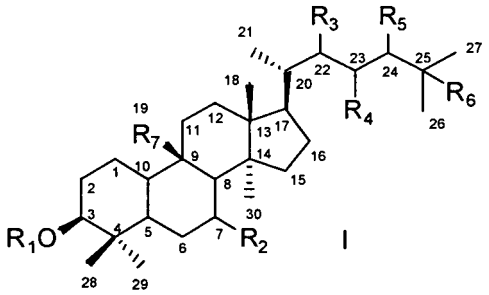
**[0003]** Now, there are over one billion overweight adults. Among them, 300 millions are suffering from obesity, and this figure tends to increase quickly, resulting in a rapid increase in obesity-related diseases, such as type 2 diabetes, cardiac diseases, stroke and hypertension. The major reasons resulting in overweight and obesity are attributed to high fatty and high calorie diet, lack of exercise and the accelerating urbanization. There are only two marketed anti-obesity drugs that can be used in a long term: one is orlistat, a specific inhibitor of gastrointestinal tract lipases, but has very common gastrointestinal-related adverse effects; the other is sibutramine, a monoamine reuptake inhibitor, but it may increase blood pressure and heart rate. So there is an unmet need to develop safe and effective weight loss drugs.

**[0004]** *Momordica charantia L.* belongs to the family Cucurbitaceae and it has been widely used in China for more than 7 hundred years as a medicinal remedy for dispelling "heat", detoxicating, improving acuity of vision, invigorating stomach, relieving thirst, stopping diarrhea and as a helminthicide. The major chemical constituents in *Momordica charantia L.* include triterpenoid saponins, cerebrosides and polypeptides. Although several reports in the literature have described a hypoglycemic effect of *Momordica charantia L* extracts, it is not known whether **cucurbitane** triterpenoids isolated from *Momordica charantia L* have similar effects to reduce hyperglycaemia. Harinantenaina et al. (Chem. Pharm. Bull 54:1017-1021, 2006) has reported that triterpene-5β, 19-epoxy-3β,25-dihydroxycucurbita-6,23(*E*)-diene and 3β,7β, 25-trihydroxycucurbita-5,23(*E*) -dien-19-al isolated from *Momordica charantia L.* showed hypoglycemic effect on alloxan-induced diabetic mice at a dose of 400 mg/kg but the effect they reported was very mild. Moreover, there is no report to indicate that these compounds can stimulate the glucose uptake in muscle and adipose cells, promote the translocation of the glucose transporter 4 (GLUT4) to the cell membrane, increase the activity of adenosine monophosphate activated protein kinase (AMPK) and thus can be used for prevention and treatment of diabetes and obesity.

### Disclosure of the Invention

**[0005]** The object of the present invention is to provide a use of cucurbitane triterpenoids represented by the following formula isolated from *Momordica charantia L.* and pharmaceutical compositions thereof in the manufacture of drugs for prevention and treatment of diabetes and obesity. More particularly, these compouds have the functions of stimulating the glucose uptake in muscle and adipose cells, promoting the tranlocation of glucose transporter 4 (GLUT4) to the cell membrane and increasing the activity of adenosine monophosphate-activated protein kinase (AMPK), and thus may have the potential for prevention and treatment of diabetes and obesity. So the cucurbitane triterpenoids isolated from *Momordica charantia L.* and pharmaceutical compositions thereof may be acted as a glucose uptake stimulator, an agonist for the translocation of glucose transporter 4(GLUT4) to the cell membrane, and an activator of adenosine monophosphate-activated protein kinase (AMPK) in muscle and adipose cells.

**[0006]** The present invention provides cucurbitane triterpenoids isolated from *Momordica charantia L.* and showing the activity of preventing and treating diabetes and obesity, which are represented by the following formula I:




wherein $R_1$ is β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; and C5 forms a double bond together with C6; C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

**[0007]** $R_1$ is β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration respectively; or

**[0008]** $R_2$ and $R_6$ are a hydroxyl group, respectively; $R_1$, $R_3$, $R_4$ and $R_5$ are each hydrogen; $R_7$ is an aldehyde group; C5 and C6, and C23 and C24 form a double bond, respectively; or

**[0009]** $R_1$ is hydrogen; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration respectively.

**[0010]** The present invention provides pharmaceutical compositions having the activity of preventing and treating diabetes and obesity, which is **characterized in that** the composition contains one or more of the cucurbitane triterpenoids isolated from *Momordica charantia L.* in a therapeutically effective amount and pharmaceutically acceptable adjuvants. The pharmaceutically acceptable adjuvants iclude, but not limited to, fillers, excipients well known in the art.

## Brief Description of Drawings

**[0011]**

Fig. 1 shows the effect of momordicoside A on the glucose uptake in L6 muscle cells;

Fig. 2 shows the effects of various compounds on the translocation of glucose transporter 4 (GLUT4) to the cell membrane;

Fig. 3 shows the effects of trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*),23(*R*), 24(*R*),25-tetrahydroxycucurbita-5-ene on the activity of adenosine monophosphate-activated protein kinase (AMPK);

Fig. 4 shows the dose dependency of 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene on the translocation of glucose transporter 4 (GLUT4) to the cell membrane and the AMPK phosphorylation in 3T3-L1 adipose cells;

Fig. 5 shows the effect of 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene on GLUT4 translocation under the condition that the PI3K/Akt insulin signaling pathway is inhibited;

Fig. 6 shows the effects of trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*),23(*R*), 24(*R*),25-tetrahydroxycucurbita-5-ene on the activity of protein kinase B (Akt).

## Best Mode for Carrying out the Invention

**[0012]** The present invention will be further described in detail with reference to the following examples and drawings, which should not be construed as the limitation for the invention.

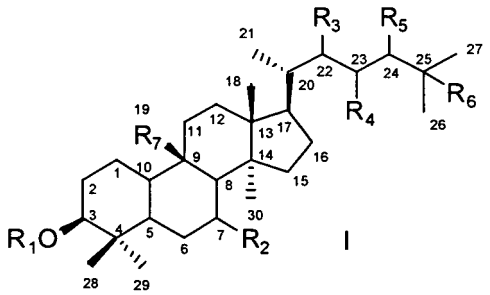
I. Summary

**[0013]** The present invention provides a use of cucurbitane triterpenoids isolated from *Momordica charantia L.* and pharmaceutical compositions thereof in the manufacture of drugs for prevention and treatment of diabetes and obesity.

**[0014]** The activity of prevention and treatment of diabetes and obesity in the invention refers to the ability to stimulate glucose uptake in muscle and adipose cells, promote the translocation of the glucose transporter 4 (GLUT4) to the cell membrane and increase the activity of adenosine monophosphate-activated protein kinase (AMPK)

□. Compounds

**[0015]** The present invention provides cucurbitane triterpenoids isolated from *Momordica charantia L.* and showing the activity of preventing and treating diabetes and obesity, which are represented by the following formula I:



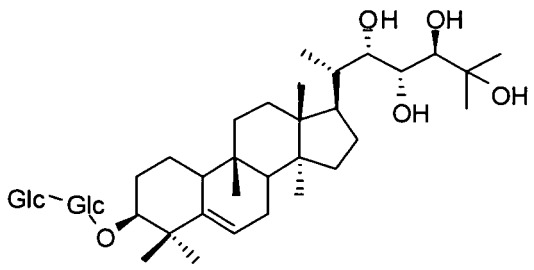

wherein $R_1$ is β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

**[0016]** $R_1$ is β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

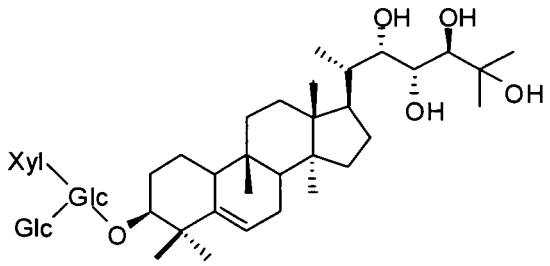
**[0017]** $R_2$ and $R_6$ are a hydroxyl group, respectively; $R_1$, $R_3$, $R_4$ and $R_5$ are each hydrogen; $R_7$ is an aldehyde group; C5 and C6, and C23 and C24 form a double bond, respectively; or

**[0018]** $R_1$ is hydrogen; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively.

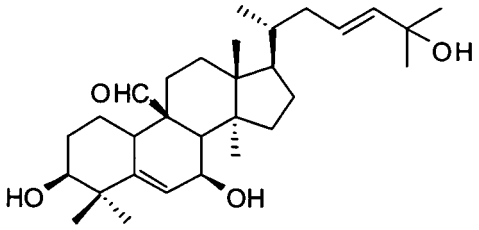
**[0019]** Specifically, these compounds include momordicoside A, momordicoside B, trihydroxycucurbita-5, 23(*E*)-dien-19-al and 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene, which are represented by the following formula, respectively:



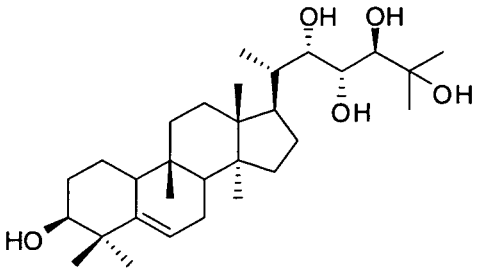

momordicoside A




momordicoside B




trihydroxycucurbita-5,23(*E*)-dien-19-al




22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene

**Examples**

**[0020]** Thin-layer chromatography (TLC) silica gel plate and silica gel (200 ~ 300 mesh) used in column chromatography

are manufactured by Qingdao Haiyang Chemical Group Corporation. The TLC spots were stained by spraying a solution of sulphuric acid-vanillin in ethanol.

**[0021]** Unless otherwise specified, all the ratios of related solvents herein are volume ratios.

## Example 1

**[0022]** The preparation of the compounds

850 kg of fresh *Momordica charantia L.* was lyophilized to afford 85 kg of dry product, which was then pulverized and macerated in a 90% ethanol aqueous solution (volume ratio) at room temperature three times, each for three days, wherein the amount of the ethanol aqueous solution is 10 times of the weight of the raw material. The three ethanol solutions obtained from the above extraction were combined, and concentrated under reduced pressure to yield a total ethanol extract. After the total ethanol extract was diluted with water (50 L), the diluted solution was partitioned with dichloromethane (20 L) to give a dichloromethane extract and an aqueous solution. Then the aqueous solution was partitioned with n-butanol (20 L) to provide 800 g of n-butanol extract. The 800 g n-butanol extract was mixed with 500 g of AB-8 type macroporous resin (manufactured by Tianjin Gelatine Plant), and the resin mixed with the sample was put on a chromatographic column loaded with 3 kg of AB-8 resin, and eluted with 12 L of pure water, 30 % ethanol (volume ratio) and 95% ethanol (volume ratio) respectively to afford 600 g of KG6, 60 g of KG7 and 80 g of KG8 respectively.

**[0023]** 80 g of KG8 was subjected to a silica gel (2 kg, 100-200 mesh) column chromatography eluting successively with a subnatant liquid (each 10 L) of chloroform-methanol-water at a volume ratio of 40:3:1, 20:3:1, 10:3:1 and 65:35:10 respectively. Each 500 ml elute was collected as one fraction, and tested on a TLC plate, wherein a mixture of chloroform-methanol (v/v = 10/1, 6/1 or 4/1) or a subnatant liquid of chloroform-methanol-water (v/v/v = 10/3/1 or 65/35/10) was used as an eluent and 5% sulphuric acid-vanillin was used as a staining agent. According to the TLC plate, the similar fractions were combined and concentrated. The fractions with a Rf of 0.3-0.4 (effluent: chloroform-methanol with a ratio of 9:1) were combined to afford component 1 and the fractions with a Rf of 0.3-0.4 (eluent: subnatant liquid of chloroform-methanol-water with a ratio of 10:3:1) were combined to afford component 8.

**[0024]** The above component 1 was subjected to a silica gel column chromatography eluting with 1000 ml of chloroform-methanol (v/v=20/1). Each 20 ml elute was collected as one fraction, and tested on a TLC plate, wherein the eluent was chloroform-methanol (v/v=10/1), and the staining agent was 5% sulphuric acid-vanillin. The eluates, which showed a spot with a Rf of about 0.4 on the TLC plate, were combined and concentrated to afford 120 mg of trihydroxycucurbita-5,23(*E*)-dien-19-al.

**[0025]** The above component 8 was subjected to a MCI column chromatography with gradient elution (1000 ml of 30%-70% methanol aqueous solution). The obtained 40% fraction were subjected to a RP-18 column chromatography with gradient elution (500 ml of 30%-60% methanol aqueous solution). Each 20 ml was collected as one fraction, and tested on a TLC plate, wherein the eluent was the subnatant liquid of chloroform-methanol-water (v/v/v=8/3/1) and the staining agent was 5% sulphuric acid-vanillin. The elutes, which showed a spot with a Rf of about 0.3 or 0.4 on the TLC plate, were combined and concentrated to afford 250 mg of momordicoside A and 300 mg of momordicoside B, respectively.

**[0026]** 40 mg of momordicoside A was treated with 0.1 M acetic acid aqueous solution for 7 days at 37 °C. The resulted product was subjected to a preparative thin-layer chromatography eluting with chloroform-methanol (v/v=5/1). The eluates with a Rf of around 0.5-0.6 were combined and concentrated to afford 10 mg of 22(*S*),23(R),24(R),25-tetrahydroxycucurbita -5-ene.

## Experimental example 1

**[0027]** Testing the effect of momordicoside A on the glucose uptake in L6 muscle cells (Fig. 1)

**[0028]** After L6 cells were differentiated to myotube completely, they were incubated in serum-free DMEM containing 0.5% BSA for 16 hours. Then momordicoside A (final concentration 50μM) was added therein to treat the cells for 1 hour and 20 minutes, while DMSO with the same volume was added into the blank control group. After that, they were washed with 1 × PBS preheated at 37 °C twice, and 0.5% BSA Krebs buffer (NaCl 140mM, KCl 5mM, $MgSO_4$ 2.5mM, $CaCl_2$ 1mM, HEPES 20mM, pH7.4) without or with insulin (final concentration 100 nM), followed by incubation at 37°C for 40 minutes. A 2-[1,2-3H(N)]-deoxy-D-glucose solution (final concentration 0.5 μCi/ml) was added therein and incubated for 10 minutes at 37°C. Then the reaction was terminated by washing three times with ice-cold 1x PBS and 0.15 ml of 0.1% Triton was added therein for the lysis of the cells the counting in a liquid-scintillation counter. After the CPM value was corrected with the protein amount, the glucose uptake amount of L6 cells was calculated.

**[0029]** The results showed that the glucose uptake in L6 cells was increased significantly both at base level and under the stimulus of insulin after treated with 10 μM momordicoside A for 2 hours (Fig. 1). The data are shown in mean ± standard error (X ± SE) (n=3). The significance was shown as *p<0.05 compared with control groups under corresponding

conditions.

**Experimental example 2**

**[0030]** Effects of isolated compounds on the translocation of glucose transporter 4 (GLUT4) from cytosol to the cell membrane (Fig.2)

**[0031]** After L6 cells were differentiated to myotube completely, they were treated for 2 hours with various test compounds, namely trihydroxycucurbita-5,23(*E*)-dien-19-al, 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene, momordicoside A, momordicoside B (final concentration 10μM, each) or 100 nM insulin (as positive control). The data were expressed as mean ± standard error (X ± SE) (n=3-4). The significance was shown as ***p<0.001 compared with solvent control groups. The results showed that trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene were capable of promoting the translocation of glucose transporter 4 (GLUT4) to the cell membrane, and hence increase the glucose uptake in cells.

**Experimental example 3**

**[0032]** Testing the activities of trihydroxycucurbita-5,23(*E*)-diene-19-aldehyde and 22(*S*), 23(*R*),24(*R*),25-tetrahydroxy cucurbita-5-ene on adenosine monophosphate-activated protein kinase (AMPK) (Fig.3)

**[0033]** 3T3-L1 adipose cells was incubated for 60 minutes in a medium containing 10 μM compounds, or in a medium containing 2 mM 5-amino4-imidazolecarboxamide nucleotide (AIC, positive control) or DMSO as a solvent control, followed by being treated with 100 nM insulin for 2 minutes or 25 minutes. Then the proteins pACC and pAMPK in cell lysate were determined with corresponding antibodies. The total amount of protein 14-3-3 was used as the quality control for sample loading. The results showed that trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene were capable of activating the AMPK signaling pathway significantly, and thus might be useful for the treatment of diabetes and obesity.

**Experimental example 4**

**[0034]** Dose-dependent effect of 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene on GLUT4 translocation and the phosphorylation of AMPK in 3T3-L1 adipose cells (Fig. 4)

**[0035]** According the processes in experimental examples 2 and 3, 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene was tested at different concentrations ($10^{-10}$, $10^{-7}$, $10^{-6}$, $10^{-5}$ M). The results were shown in Fig. 4, wherein A was the dose dependency curve regarding the effect on translocation of glucose transporter 4(GLUT4); B showed the effect of the compound at different concentrations on the activity of adenosine monophosphate-activated protein kinase(AMPK) in 3T3-L1 adipose cells; C showed the quantification of the ratio of phosphorylated adenosine monophosphate-activated protein kinase (pAMPK) to total adenosine monophosphate-activated protein kinase (tAMPK) at different concentrations of the compound in 3T3-L1 adipose cells. The experimental results showed that the effect of 22(*S*), 23(*R*), 24(*R*),25-tetrahydroxycucurbita-5-ene on the translocation of glucose transporter 4(GLUT4) to the cell membrane is significantly related to the phosphorylation of AMPK (namely, the activation of the AMPK signaling pathway), and the maximal effects was reached at the concentration of $10^{-6}$ M.

**Experimental example 5**

**[0036]** Testing the effects of 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene on the insulin signaling pathway (namely monophosphoinositide 3-kinase/protein kinase B(PI-3K/Akt) pathway) in 3T3-L1 adipose cells and L6 muscle cells.

**[0037]** A. 10 μM 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene or 100 nM insulin (positive control) was added in the differentiated 3T3-L1 adipose cells using DMSO (final concentration of DMSO: 0.2%) as a solvent with or without wortmanin (an inhibitor of monophosphoinositide 3-kinase), and the activity on GLUT4 translocation was determined as described in experimental example 2. The data were expressed in mean ± standard error (X ± SE). The significances were shown as * p<0.05 and **p<0.01 compared with the solvent control groups. The results showed that wortmanin, the inhibitor of PI3-kinase, did not affect the activity of 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene on GLUT4 translocation, indicating that the effect of 22(*S*),23(*R*),24(*R*),25- tetrahydroxycucurbita-5-ene on GLUT4 translocation is independent of this insulin signaling pathway (Fig.5).

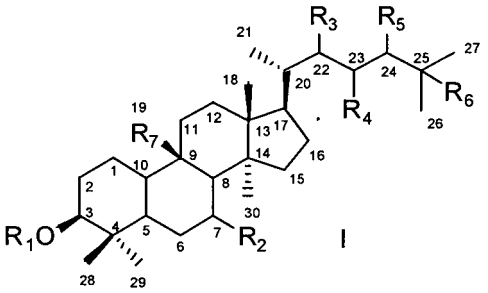
**[0038]** B. 3T3-L1 adipose cells and L6 muscle cells were incubated for 30 minutes in a medium containing 10μM trihydroxycucurbita-5,23(*E*)-dien-19-al or 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene, or a medium containing 100 nM insulin (positive control) or DMSO as a solvent control. The phosphorylation of protein kinase B [Akt (S473)] and the total protein kinase B (14-3-3) were determined with corresponding antibodies. The significances are shown as

*p<0.05 and **p<0.01 compared with the solvent control groups. The results showed that trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*), 23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene were not able to increase the phosphorylation of protein kinase B, indicating that trihydroxycucurbita-5,23(*E*)-dien-19-al and 22(*S*),23(*R*), 24(*R*),25-tetrahydroxycucurbita-5-ene did not influence the insulin signaling pathway (Fig.6).

**[0039]** The insulin signaling pathway and AMPK signaling pathway are the two main signaling pathways to mediate GLUT4 translocation and glucose uptake, and the results in Fig.5 and Fig.6 were consistent with those in Fig.3 and Fig. 4, which indicated that the compounds stimulate GLUT4 translocation and glucose uptake by the AMPK signaling pathway.

## Claims

1. A use of cucurbitane triterpenoid compounds isolated from *Momordica charantia L.* for preparing medications for prevention and treatment of diabetes and obesity with the following representative formula I:



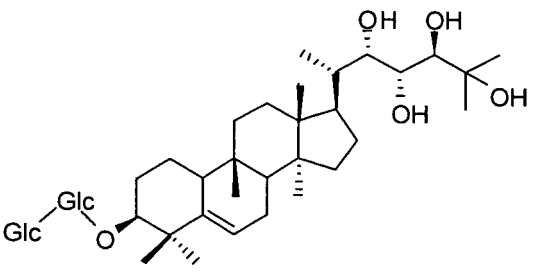
wherein $R_1$ is β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration respectively; or

$R_1$ is β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl (1→6)]β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

$R_2$ and $R_6$ are a hydroxyl group, respectively; $R_1$, $R_3$, $R_4$ and $R_5$ are each hydrogen; $R_7$ is an aldehyde group; C5 and C6, and C23 and C24 form a double bond, respectively; or

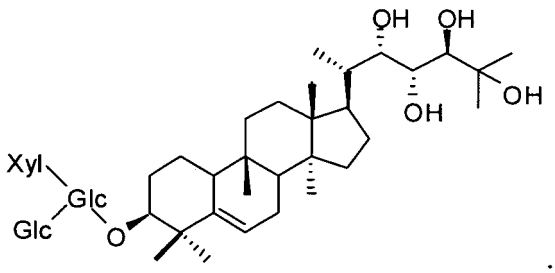
$R_1$ is hydrogen; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration respectively.

2. The use according to claim 1, wherein the cucurbitane triterpenoid compound is momordicoside A represented by the following formula

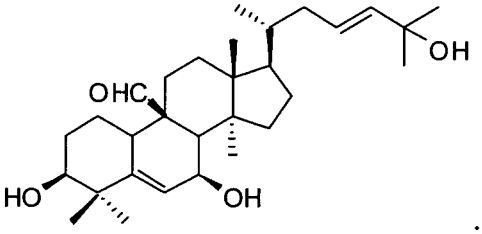


3. The use according to claim 1, wherein the cucurbitane triterpenoid compound is momordicoside B represented by

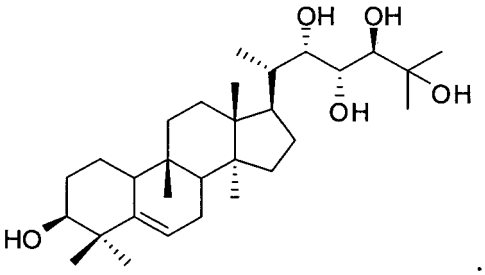
the following formula :




**4.** The use according to claim 1, wherein the cucurbitane triterpenoid compound is trihydroxycucurbita-5,23(*E*)-dien-19-al represented by the following formula :

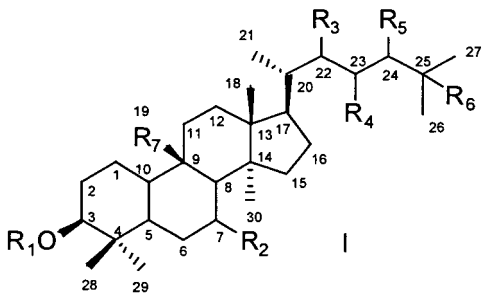



**5.** The use according to claim 1, wherein the cucurbitane triterpenoid compound is 22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbita-5-ene represented by the following formula :




**6.** The use according to any one of claims 1 to 5, wherein the cucurbitane triterpenoid compound isolated from *Momordica charantia L.* is acted as a glucose uptake stimulator , an agonist for the translocation of glucose transporter 4 to the cell membrane, and an activator of adenosine monophosphate-activated protein kinase.

**7.** A pharmaceutical composition having the activity of preventing and treating diabetes and obesity, wherein, the composition contains one or more of the cucurbitane triterpenoids represented by the following formula and isolated from *Momordica charantia L.* in a therapeutically effective amount; and pharmaceutical acceptable adjuvants:



I

wherein $R_1$ is β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

$R_1$ is β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl (1→6)]β-D-glucopyranosyl; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively; or

$R_2$ and $R_6$ are a hydroxyl group, respectively; $R_1$, $R_3$, $R_4$ and $R_5$ are each hydrogen; $R_7$ is an aldehyde group; C5 and C6, and C23 and C24 form a double bond, respectively; or

$R_1$ is hydrogen; $R_2$ is hydrogen; $R_3$, $R_4$, $R_5$ and $R_6$ are each a hydroxyl group; $R_7$ is methyl; C5 forms a double bond together with C6; and C22, C23 and C24 are chiral carbon atoms with S-, S- and R-configuration, respectively.

Fig. 1

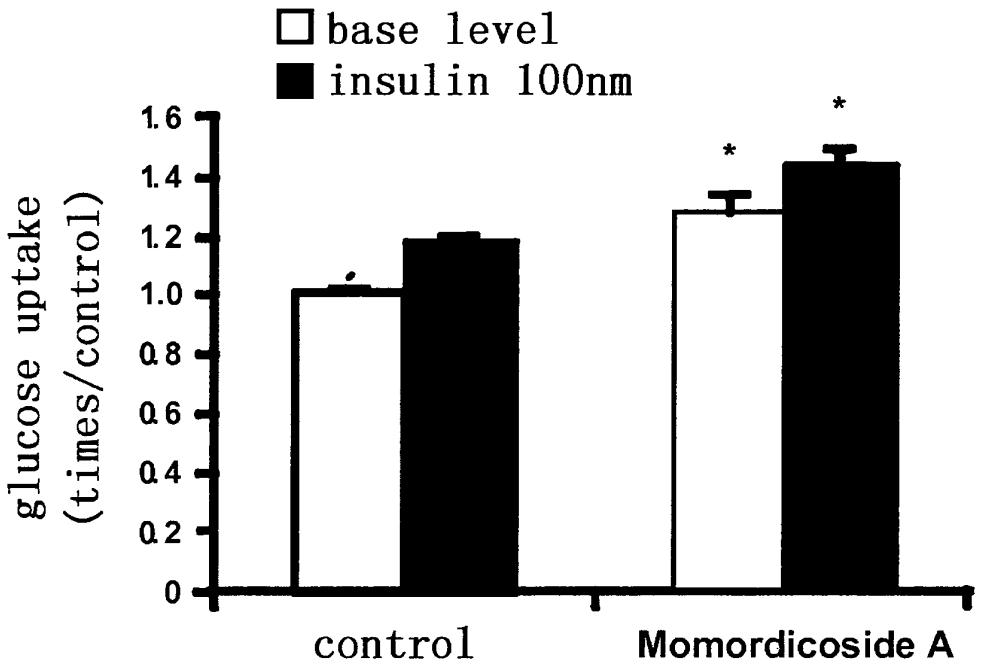
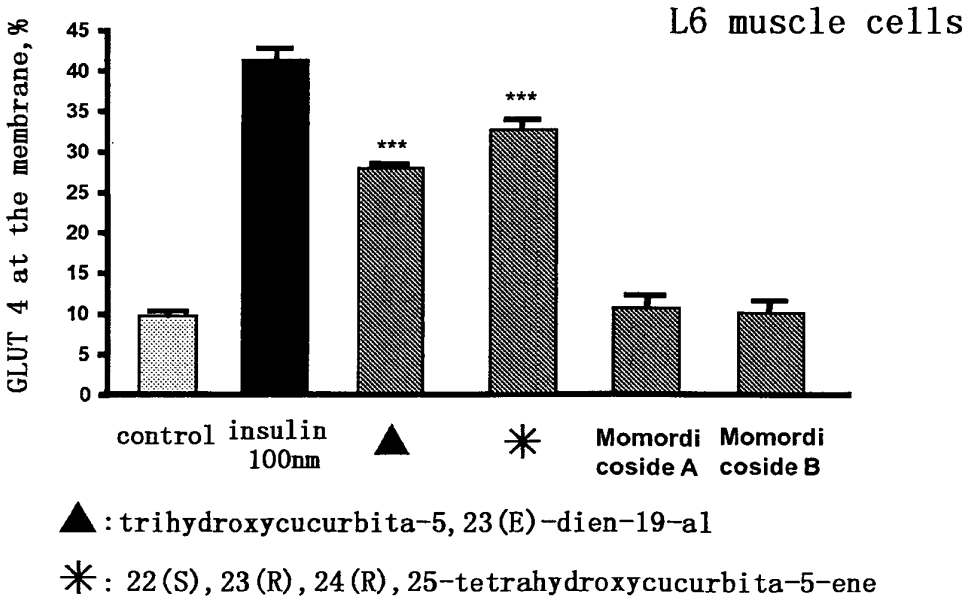


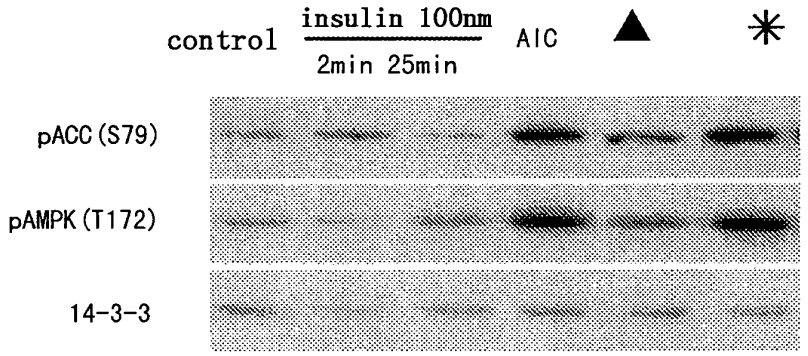

Fig.2




▲ : trihydroxycucurbita-5, 23(E)-dien-19-al

✳ : 22(S), 23(R), 24(R), 25-tetrahydroxycucurbita-5-ene

Fig.3




▲ : trihydroxycucurbita-5,23(E)-dien-19-al

✳ : 22(S),23(R),24(R),25-tetrahydroxycucurbita-5-ene

Fig.4

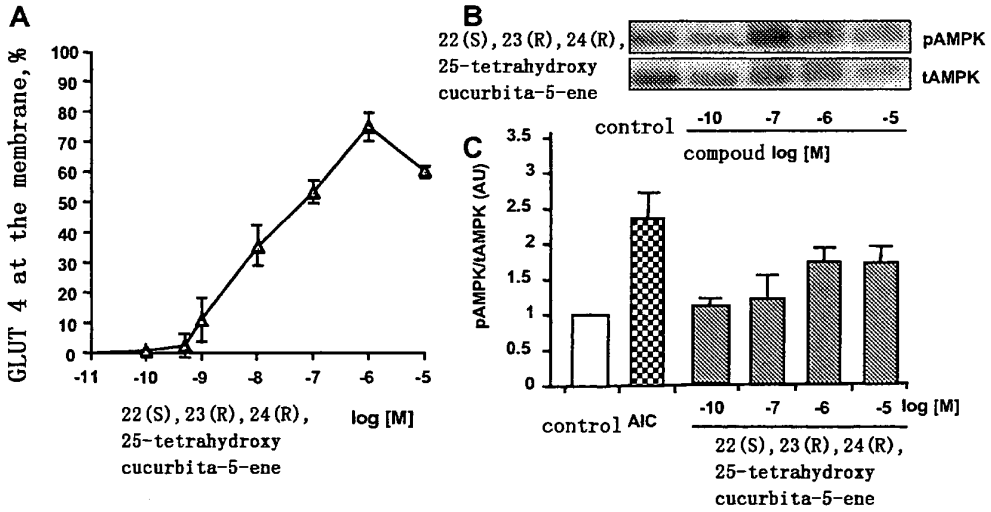


Fig.5

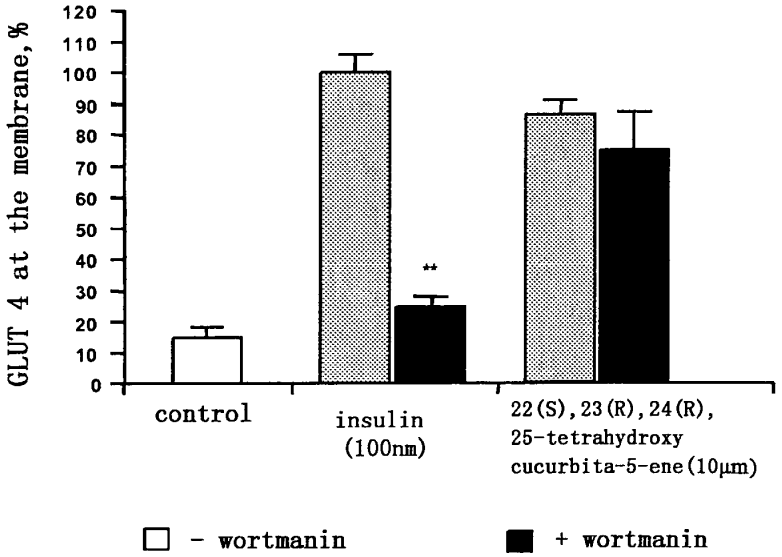
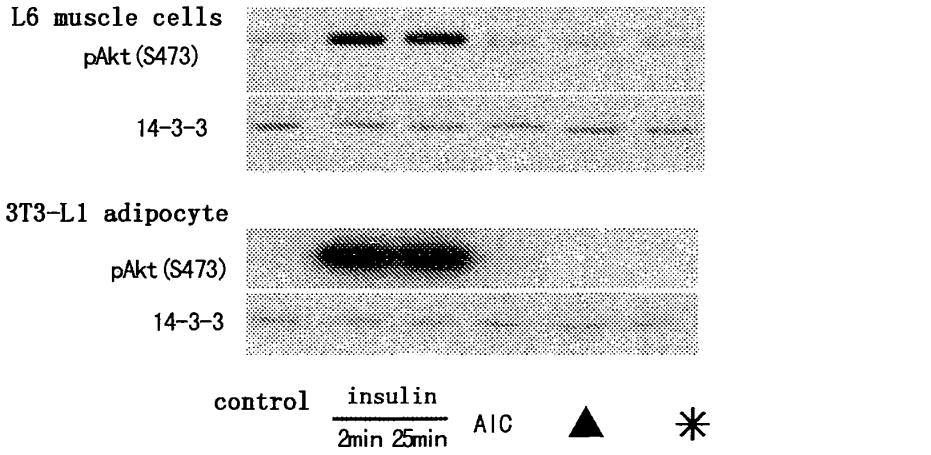

GLUT 4 at the membrane,%
120
110
100
90
80
70
60
50
40
30
20
10
0
**
control
insulin
(100nm)
22(S),23(R),24(R),
25-tetrahydroxy
cucurbita-5-ene(10μm)
- wortmanin
+ wortmanin

Fig.6


L6 muscle cells
pAkt(S473)
14-3-3
3T3-L1 adipocyte
pAkt(S473)
14-3-3
control
insulin
2min 25min
AIC
▲: trihydroxycucurbita-5,23(E)-dien-19-al
✳: 22(S), 23(R), 24(R), 25-tetrahydroxycucurbita-5-ene

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/000050

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI,EPODOC,PAJ,CNPAT(CN), CNKI(CN), Chinese Pharmaceutical Abstract (CN), CHEMICAL ABSTRACTS(US), EMBASE, STN: momordicoside, momordica charantia, balsampear fruit, bitter gourd, karela, momordica charantia, diabetes, blood glucose, hypoglycaemic, momorditine, charntin, fat, adipositas, adiposity, obesitas, obesity, loss weight, weight reduction, weight, triterpene

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | Liva H.et al. Momordica charantia constituents and antidiabetic screening of the isolated major compounds. Chem. Pharm. Bull. July 2006, Vol. 54, No. 7, page 1017 right column lines 15-17, compound 5 in page 1018 right column, page 1019 right column | 1, 4, 6, 7 |
| X | ZHANG Bingzhen et al. Modern research progress of bitter melon. Food and Drug (Chinese). 2006, Vol. 8, No. $04_A$, page 27 paragraph 1.1, page 28 paragraph 2.1, page 29 paragraph 2.5 | 1-3, 6, 7 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 08 Apr. 2009(08.04.2009) | **23 Apr. 2009 (23.04.2009)** |
| Name and mailing address of the ISA/CN<br>The State Intellectual Property Office, the P.R.China<br>6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088<br>Facsimile No. 86-10-62019451 | Authorized officer<br>XIUWEN<br>Telephone No. (86-10)62411205 |

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## INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/000050

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | LI Jian et al. The research progress on momordicoside. Food research and development (Chinese). June 2005, Vol. 26, No. 3, page 22 line 3, page 23 paragraph 2.1 | 1-3, 6, 7 |
| A | | 5 |

Form PCT/ISA/210 (continuation of second sheet ) (April 2007)

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2009/000050

CLASSIFICATION OF SUBJECT MATTER :

A61K 31/704 (2006.01) i
A61K 31/575 (2006.01) i
A61P 3/04 (2006.01) i
A61P 3/10 (2006.01) i

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## REFERENCES CITED IN THE DESCRIPTION

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### Non-patent literature cited in the description


- **Harinantenaina et al.** *Chem. Pharm. Bull,* 2006, vol. 54, 1017-1021 **[0004]**